

specifically in the *C. elegans* male and hermaphrodite germline, where it associates with P granules. P granules are mRNA/protein complexes important for germ cells function and preservation. *vbh-1(RNAi)* animals had reduced offspring due to embryo lethality and gamete abnormalities. Fifty percent of *vbh-1(RNAi)* animals did not produce sperm or failed to complete spermatogenesis. Also they initiated oogenesis prematurely during the L4 larval stage. This data suggest that VBH-1 is important for the correct sperm/oocyte switch. To understand the function of VBH-1, we made a two-hybrid screening to isolate proteins that interact with this RNA helicase. We found three proteins that interact with VBH-1, which we have called VIP for VBH-1 Interacting Protein. VIP-1 was isolated several times in the two-hybrid screening and encodes a novel RNA binding protein. VIP-2 encodes a nucleic acid binding protein and VIP-3 is a novel protein with unknown function. We are studying the role of these proteins in the *C. elegans* germline using RNA interference.

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#### Program/Abstract # 252

##### **In search of proteins that regulated starvation-induced germ cell apoptosis in *C. elegans***

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Germ cell apoptosis takes place in several organisms including mammals. Fifty percent of germ cells in *C. elegans* are eliminated during oogenesis to maintain gonad homeostasis. This germ cell apoptosis is regulated by unknown mechanisms. Higher levels of apoptosis can be triggered by DNA damage or pathogen infections. The BH3-only domain protein EGL-1 participates in both types of apoptosis, but DNA damaged-induced apoptosis is also dependent of the transcriptional factor p53 (CEP-1). Unexpectedly, we found that other types of stress that also induce germ cell apoptosis, like heat shock, oxidative, osmotic and starvation stress, are regulated independently of EGL-1 and p53. We found that heat shock, osmotic and oxidative stress are induced by the MAPKK, MEK-1 and SEK-1. However, these proteins are not necessary to induce apoptosis by starvation. To find out genes that control starvation-induced germ cell apoptosis, we compared mRNA from starved and well-fed animals using a *C. elegans* germline-specific microarray. We found nearly 200 genes whose expression levels changed during starvation. By RNA interference we found nine genes required to induce germ cell apoptosis under starvation, and two genes that protect germ cells from starvation-induced apoptosis.

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#### Program/Abstract # 253

##### **The regulation of germ cell sex determination in *Drosophila***

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Sex-specific development of the germ cells is critical for the formation of male or female gametes and the continuation of a species. However, little is known about the establishment and maintenance of germ cell sexual identity. To identify genes involved in germline sex determination and development, a molecular screen was conducted looking for genes expressed sex-specifically in the embryonic germ cells. From this screen, eight genes were found to be expressed sex-specifically in the male germ line. The expression pattern for these genes indicates that germ cell sexual identity is established by embryonic stage 15. We are using these genes to investigate how somatic signals and germ cell autonomous cues control germ cell sex determination. By changing the sex of the soma (using *transformer*) we have shown that male-specific gene expression is both induced by the male soma and repressed by the female soma. The sex chromosome constitution also affects male-specific gene expression and we are currently studying how genes known to affect germ cell autonomous sexual identity (e.g. *ovo* and *ovarian tumor*) affect initial germline sexual identity. Lastly, we are investigating the role of these genes in germ cell development. Viable mutant alleles of one of our genes, *unloaded*, affect fertility in both sexes and males show a severe depletion of germline stem cells. Since *unloaded* is expressed in male germ cells at the time that germline stem cell identity is being established in the late embryo, we are examining the role this gene may play in this process.

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#### Program/Abstract # 254

##### **Redox regulation of germ cell migration in *Drosophila***

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*Drosophila* germ cells are specified temporally and spatially separate from the somatic cells of the gonad and therefore must migrate to associate with them. In a reverse genetic screen for genes that alter *Drosophila* germ cell migration, we identified a null mutation in the thioredoxin peroxidase gene, *Jafrac1*, with a striking germ cell internalization defect. During normal gastrulation primordial germ cells (PGCs) associate tightly with the invaginating midgut primordium. In contrast, in *Jafrac1* mutant embryos the association of germ cells with the midgut is lost and some germ cells are left outside of the embryo. Previously, germ cells were thought to be passively swept into the gut during gastrulation, however our data suggest that this is an active and genetically tractable process. In addition, overexpression of *Jafrac1* protein in germ cells is sufficient to cause precocious transepithelial migration of PGCs through the midgut primordium. *Jafrac1* is one of three dual cysteine thioredoxin

peroxidases in the *Drosophila* genome. These enzymes act as acceptors for hydrogen peroxide and promote its reduction to water. Orthologs of Jafrac1 have been implicated in modulating signaling in yeast and mammalian cell culture. Our results suggest that redox-mediated signaling regulates germ cell migration.

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#### Program/Abstract # 255

##### **IGF signaling cell-autonomously promotes primordial germ cell migration in zebrafish**

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Whole-body suppression of insulin-like growth factor receptors (IGF1R) in zebrafish embryos results in reduced somatic growth, in addition to mismigration and elimination of primordial germ cells (PGCs). As PGCs rely upon migratory guidance cues from somatic cells, it is unclear whether the PGC defect in IGF1R-deficient embryos stems from defective somatic development, or whether PGCs cell-autonomously require IGF signaling for migration. We hypothesized that PGCs are cell-autonomously dependent upon IGF signaling for migration, based upon the chemotactic response of other cell types to IGF signaling. To test this hypothesis, we designed an expression construct encoding a dominant-negative IGF1R (dnIGF1R) fused to the 3' untranslated region (3'UTR) of the zebrafish *nanos1* gene (*nos1*); the latter element prevents degradation of associated mRNA sequences in germ cells, but permits rapid degradation in somatic cells. This construct thus provides a means to suppress IGF1R function in PGCs while retaining normal IGF signaling in the soma. In accordance with our predictions, zebrafish embryos expressing dnIGF1R:*nos1* exhibited normal somatic development, but had significantly fewer PGCs colonizing the genital ridges, and a corresponding increase in the number of mismigrated (ectopic) PGCs. These data support the hypothesis that PGCs in zebrafish are cell-autonomously dependent on signaling through IGF1R for migration to the genital ridges.

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#### Program/Abstract # 256

##### **Induced sexual maturation in eel with embryonic zebrafish cell lines that constitutively produce LH and FSH**

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Many fish species mature in response to environmental conditions. Two isoforms of gonadotropin-releasing hormones have

been found in teleost species: the follicular stimulating hormone (FSH) and the luteinizing hormone (LH). FSH controls gonadal development and the early stages of gametogenesis and LH controls the final maturation stages. In aquaculture maturation is often artificially stimulated by regular injections with pituitary extracts. However, the intermittent injections cause large fluctuations in the circulating hormone levels and the regular injections cause major stress effects on the animal. Thus, minimising handling and particularly initiating maturation in a natural way will improve the reproduction success. The aim of this project is to develop a novel method to improve maturation of eggs using cells that produce constitutively LH and FSH. The hormone producing cells were transplanted into eels to provide a continuous source for the hormone, thereby reducing the need for regular injections. The presence of the cells was monitored weekly during 1 month. We were able to induce maturation shown as changes in the morphology of eyes and pectoral fins. We developed new molecular probes for testing induction of maturation. The results of the qRT-PCR showed an increase in expression of vitellogenin genes, characteristic of the final maturation steps; these findings suggest a breakthrough towards fish reproduction. Work supported by European Commission 6th Framework Programme grant (LSHG-CT-2003-503496, ZF-Models).

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#### Program/Abstract # 257

##### **Identification of the RCK/p54/Cgh-1 homolog in zebrafish**

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Germ cells are specified early during development. Oocyte determination in zebrafish and other organisms is induced by the accumulation of maternal determinants in germ granules. After fertilization and during early cell divisions these determinants, which are proteins, mRNA and mitochondria, are segregated to only those cells that will give rise to the germline. Many proteins in germ granules have RNA binding domains. RCK/p54/Cgh-1 is a DEAD box RNA helicase highly conserved and present in germ granules from *C. elegans* to humans. This helicase regulates mRNA translation by maintaining maternal mRNA masked, it is also involved in cell cycle progression in yeast and germ cell apoptosis protection in *C. elegans*. RCK is an essential gene for *Drosophila* and in mammals it is found in the germ line as well in P-bodies from somatic cells, which is the place where the RNAi pathway takes place. We found in the zebrafish genome two RCK/p54/Cgh-1 homologs in chromosomes 18 and 16 that we named RCKa and RCKb respectively. We have cloned RCKa and we are in the process of cloning RCKb. RT-PCR experiments showed that RCKa is expressed maternally in oocytes and also through all developmental stages from two cells to 5 days post-